UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: August 15, 2001

MEMORANDUM

SUBJECT: **PROPANIL**: Report of the Hazard Identification Assessment Review Committee

FROM: Yung G. Yang, Ph.D.
And
Susan L. Makris, M.S.
Toxicology Branch
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Richard Griffin
Risk Assessor, RRB2
Health Effects Division (7509C)

PC CODE: 028201

On July 19, 2001, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) convened to review the toxicology data base of propanil for hazard identification and to select doses and endpoints for acute dietary, chronic dietary (RfD) as well as occupational and residential exposure assessments and to address the sensitivity of infants and children from exposure to propanil as required by the Food Quality Protection Act (FQPA) of 1996. The HIARC's conclusions are presented in this report.

Committee Members in Attendance

Members in attendance: Ayaad Assaad, William Burnam, Jonathan Chen, Elizabeth Doyle (co-Chair), Elizabeth Mendez, Jess Rowland (co-Chair), David Nixon, and Yung Yang.

Members in absentia: Pamela Hurley, and Brenda Tarplee.

Also, in attendance: Paula Deschamp, Alberto Protzel, Shanna Recore, and Pauline Wagner

Data evaluation / preparation: Robert Fricke and Susan Makris

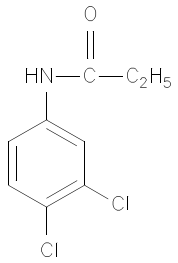
Data presentation: _____

Yung G. Yang
Toxicology Branch.

INTRODUCTION

Propanil (N-(3,4-dichlorophenyl)-propanamide) belongs to the acetanilide class of pesticides. Propanil is a contact-type, post-emergence herbicide used to control grasses and broad-leaved weeds, primarily in rice. Available formulations include emulsifiable concentrates, liquid and dry flowable, low volume, and ultra low volume formulations. There are no residential uses.

Empirical formula:	C_9H_9ClNO
Molecular weight:	218.09
CAS Registry No.:	709-98-8
PC Code:	028201



At a previous meeting, held on May 31, 2001, the HIARC had decided to defer final decisions on propanil until such time that additional information were available to upgrade the developmental toxicity studies in rats and rabbits.

On May 9, 2001 the HED Cancer Peer Review Committee (CARC) evaluated the carcinogenic potential of propanil and determined that the data indicate "suggestive evidence of carcinogenic potential by all routes of exposure, but not sufficient to assess human carcinogenic potential".

On July 19, 2001 the HIARC reviewed the updated toxicology data base of propanil for hazard identification and to select doses and endpoints for acute dietary, chronic dietary (RfD) as well as occupational and residential exposure assessments in support of a RED and to address the sensitivity of infants and children from exposure to propanil as required by the Food Quality Protection Act (FQPA) of 1996. The HIARC's conclusions are as follows.

1. HAZARD IDENTIFICATION

1.1 Acute Reference Dose (RfD)

Study Selected: None

MRID No.: N/A

Executive Summary: N/A

Dose and Endpoint for Establishing RfD: N/A

Uncertainty Factor (UF): N/A

Comments about Study/Endpoint/Uncertainty Factor: No appropriate effects attributed to a single exposure (dose) were identified in any study including the rat or rabbit developmental toxicity studies. The prenatal developmental toxicity studies were examined for possible endpoints that could be used in acute dietary risk assessment for the general population and for females aged 13-50. In the rat developmental toxicity study, body weight loss was observed in dams at 100 mg/kg/day after only 4 gavage doses of propanil (NOAEL = 20 mg/kg/day). A similar effect was noted in the rabbit developmental toxicity study, in which body weight loss was observed in does following 6 daily gavage doses at 100 mg/kg/day (NOAEL = 20 mg/kg/day). In each study, this was the first scheduled post-treatment body weight measurement, however, there was insufficient evidence that these findings were the result of a single dose. Body weight decrements observed during the first week of treatment in the chronic toxicity study in the rat were considered, but were not used in selecting an acute RfD, since the first observation was at 7 days of treatment and since the contribution of palatability issues to this effect can not be assessed. Additionally, in the rabbit developmental study the dose-response nature of the decrease in fetal body weight was not apparent at the mid-dose level, there were no skeletal ossification delays to support the fetal weight deficits, and fetal weight decrements at the high dose were observed in the presence of severe maternal toxicity. Based upon these attributes, there was not a high degree of confidence in the use of this endpoint for acute dietary risk assessment for females 13-50. Therefore, hazard was not identified for quantitative risk assessment.

Acute RfD = N/A

1.2 Chronic Reference Dose (RfD)

Study Selected: Chronic toxicity/carcinogenicity study- rats Guideline #: 870.4300

MRID No.: 43303201

Executive Summary: Randomized groups of 70/sex/dose Sprague-Dawley rats were fed diets containing 0, 200, 600, or 1800 ppm of technical Propanil (96.5-99.5% a.i) (9.0, 27.7, 88 mg/kg/day, males; 11.5, 38.3, 145 mg/kg/day, females) for 104 weeks (MRID 43303201). 20/sex/dose were sacrificed after 52 weeks. All rats were examined daily for toxicity and mortality and weekly for detailed clinical findings. Clinical pathology data were taken at 13, 26, 52, 78, and 104 weeks. All sacrificed animals were necropsied and organ weights were taken at 52 and 104 weeks. Histopathological examination was conducted on all animals which died on study or were sacrificed at 52 or 104 weeks.

At 200 ppm, the following treatment-related effects were observed: statistically significantly increased methemoglobin at weeks 13, 26, and 52, ranging from 33-45% above control levels, significantly decreased packed cell volume and red blood cells at weeks 26 and 52 ranging from 4-6%, significantly increased absolute weight of spleen (14%) in females at 52 weeks, enlarged spleens in 104 week necropsied females, small seminal vesicles and prostates in 104 week necropsied males, hemosiderosis in spleen of males, brown pigment (probably hemosiderin) in proximal convoluted tubules of females and 8% incidence of endometrial polyps in females (compared to 4% in controls).

At 600 ppm, the following treatment-related effects were observed: decreased weight gains ranging from 7-15% in males and 24-32% in females (excluding the 41% decrease in males and 47% decrease in females during the 1st week), decreases in food consumption ranging from 5-7% in males and 1-6% in females, increases in food efficiency in males and females (17.9 ♂and 18.6 ♀) in comparison to controls (16.7% ♂ and 10.5 % ♀), decreases in packed cell volume ranging from 7.6-12.5% at all sampling intervals in females, decreases in hemoglobin ranging from 7.5-13.5% at all sampling intervals in males, decreases in red blood cells ranging from 9.5-11.3% at all sampling intervals in females, increases in methemoglobin ranging from 61-119% at all sampling intervals in females, increases in methemoglobin ranging from 31-62% at all sampling intervals in males, significant increases in BUN and decreases in triglycerides at weeks 26 and 52 in males, increases in BUN at all sampling intervals and decreases in triglycerides at weeks 52 and 78 in females, significantly increased absolute and relative spleen weights for males and females at weeks 52 and 104, enlarged spleens at week 104 in both sexes, testicular masses at week 104, macrophage aggregations in the mesenteric lymph nodes (F), centrilobular liver cell enlargement (M), hepatic granulomatous inflammation (F), pericholangitis (M,F), brown pigment in Kupffer cells (M,F), convoluted tubule epithelium (M), sperm absent in epidydimides, reduced secretion from seminal vesicles, 16% incidence of benign interstitial cell tumors in testes of males (6% in controls) and 13 %

incidence of endometrial polyps in females.

At 1800 ppm, the following treatment-related effects were observed: discoloration of upper and lower incisors in females, increased survival in both sexes (62% ♂, 66% ♀ in comparison to controls: 30% ♂, 38% ♀; p = 0.013 ♂, 0.010 ♀), decreased weight gain ranging form 24-30% in males and 27-65% in females, decreased food consumption ranging from 11-16% in males and 2-5% in females, food efficiency increases of 20.2% for males and 50.3% for females, decreased packed cell volume ranging from 6-9% in males and 13-22% in females, decreased hemoglobin ranging from 8-15% in males and 15-22% in females, decreased RBC ranging from 9-15% in males and 18-23% in females, increased methemoglobin ranging form 58-64% in males and 106-196% in females, increased bilirubin in males at weeks 26, 52, and 104, increased BUN and decreased triglycerides at weeks 26 and 52 in males, increased bilirubin and BUN at all sampling intervals in females, decreased triglycerides at weeks 52 and 788 in females, increased absolute and relative spleen weights in both sexes at weeks 52 and 104, enlarged and darkened spleens in both sexes at weeks 52 and 104, liver masses in females, testicular masses in males, broken incisors in both sexes at week 52 and 104, ovarian cysts and thickened uteri in females at week 104, macrophage aggregations in the mesenteric lymph nodes (M), hemosiderosis of the spleen (F), hepatic centrilobular enlargement (F), hepatic granulomatous inflammation (M), focal interstitial cell hyperplasia with marked tubular atrophy, atrophy of prostate, galactoceles in mammary gland, azonal degeneration of sciatic nerve, dilatation of uterus, cystic ovarian bursa, 58% incidence of benign interstitial cell tumors in testes of males, 12% incidence of hepatocellular adenomas in females, 13% incidence of endometrial polyps in females. The incidence of testicular interstitial cell adenomas in the male historical controls ranged from 1-11% and the historical control range for female hepatocellular adenomas ranged from 0-2%.

A NOAEL was not established in this study for systemic effects due to the findings at 200 ppm (LDT).

This chronic toxicity /carcinogenicity study in rats is classified as Acceptable/Guideline.

Dose and Endpoint for Establishing RfD: 9 mg/kg/day (LOAEL) based on increased methemoglobin and increased spleen weight in females, and observations of small seminal vesicles and prostates in males.

Uncertainty Factor(s): 300

Comments about Study/Endpoint/Uncertainty Factor: An additional UF of 3 is applied for the use of a LOAEL. A one-year dog study (MRID 42962901) with a LOAEL of 5 mg/kg/day was considered. However, this study was not selected because the observed effects were considered minimal and the toxicity could have been enhanced due to

enterohepatic circulation.

Chronic RfD = 9.0 (LOAEL) mg/kg/day / 300 (UF) = **0.03 mg/kg/day**

1.3 Occupational/Residential Exposure

1.3.1 Short-Term (1day to 30 days) Incidental Oral Exposure

Study Selected: Chronic toxicity/carcinogenicity study- rats

MRID No.: 43303201

Executive Summary: See chronic RfD.

Dose and Endpoint for Risk Assessment: 9 mg/kg/day (LOAEL) based on increased methemoglobin. A NOAEL was not established.

Comments about Study/Endpoint: Methemoglobinemia is the principal toxicological effect of concern for propanil. This effect was seen at week 13 (the first measurement period) in rats and dogs. There are no data for earlier time points (i.e., days 1-89); therefore, this endpoint/dose at the 13-week observation period is selected for this exposure scenario.

1.3.2 Intermediate-Term (1 month to Several Months) Incidental Oral Exposure

Study Selected: Chronic toxicity/carcinogenicity study- rats

MRID No.: 43303201

Executive Summary: See Chronic RfD.

Dose and Endpoint for Risk Assessment: 9 mg/kg/day (LOAEL) based on increased methemoglobin. A NOAEL was not established.

Comments about Study/Endpoint: Methemoglobinemia is the principal toxicological effect of concern. This effect was seen at week 13 (the first measurement period) in rats and dogs. The endpoint/dose observed at the 13 week observation period is appropriate for this exposure scenario.

1.3.3 Dermal Absorption

No dermal absorption study is available.

Dermal Absorption Factor: An estimation of a dermal absorption factor of 20% is extrapolated using the maternal LOAEL of 100 mg/kg/day from the developmental toxicity study in rabbits (MRID 00058589) and the LOAEL of 500 mg/kg/day from the 21-day dermal study in rabbits (MRID 41777001, 41961800): the ratio is 100/500 or 20%. This dermal absorption factor is supported by a dermal absorption study with linuron, a structurally-related acetanilide, in which a 16% of dermal absorption was observed over an eight hour exposure (HED Doc No. 01346, Dated 12/7/94).

1.3.4 Short-Term Dermal (1 day to 30 days) Exposure

Study Selected: Chronic toxicity/carcinogenicity study- rats

MRID No.: 43303201

Executive Summary: See chronic RfD.

Dose and Endpoint for Risk Assessment: 9 mg/kg/day (LOAEL) based on increased methemoglobin. A NOAEL was not established.

Comments about Study/Endpoint: Methemoglobinemia is the principal toxicological effect of concern. This effect was seen at week 13 (the first measurement period) in rats and dogs. There are no data for earlier time (i.e., days 1-89); therefore, this endpoint/dose was selected for this exposure scenario. The 21-day dermal toxicity study in rabbits (MRID 41777001, 41961800) was not selected because the methemoglobinemia was not measured in this study and this effect was seen in three other species (mice, rats, and dogs). The selection of the 9 mg/kg/day (LOAEL) is a conservative regulatory endpoint which will yield an upper-bound risk assessment. Since an oral dose was selected route-to-route extrapolation is required with a dermal absorption factor of 20%.

1.3.5 Intermediate-Term Dermal (1 month to 6 Months) Exposure

Study Selected: Chronic toxicity/carcinogenicity study- rats

MRID No.: 43303201

Executive Summary: See chronic RfD.

Dose/Endpoint for Risk Assessment: 9 mg/kg/day (LOAEL) based on increased

methemoglobin. A NOAEL was not established.

Comments about Study/Endpoint: Methemoglobinemia is the principal toxicological effect of concern. This effect was seen at week 13 (the first measurement period) in rats and dogs. This endpoint/dose is appropriate for this exposure scenario. The 21-day dermal toxicity study in rabbits was not selected because the methemoglobinemia was not measured in this study and this effect was seen in three other species (mice, rats, and dogs). The selection of the 9 mg/kg/day (LOAEL) is a conservative regulatory endpoint which will yield an upper-bound risk assessment. Since an oral dose was selected route-to-route extrapolation is required with a dermal absorption factor of 20%.

1.3.6 Long-Term Dermal (Longer than 6 Months) Exposure

Study Selected: Chronic toxicity/carcinogenicity study- rats

MRID No.: 43303201

Executive Summary: See chronic RfD.

Dose and Endpoint for Risk Assessment: 9 mg/kg/day (LOAEL) based on increased methemoglobin and increased spleen weight in females, and observations of small seminal vesicles and prostates in males.

Comments about Study/Endpoint: The endpoint/dose is appropriate for this exposure scenario. An additional UF of 3 is applied to account for the lack of a NOAEL in this study. Since an oral dose was selected route-to-route extrapolation is required with a dermal absorption factor of 20%.

Inhalation Exposure

Except for an acute inhalation study, for which propanil was placed in Toxicity Category IV (LC_{50}>6.1 mg/L), no other studies are available via this route. Therefore, the HIARC selected the NOAELs from oral studies for risk assessment. Since the doses identified for inhalation risk assessment are from oral studies, route-to-route extrapolation should be as follows:

> The inhalation exposure component (i.e., µg a.i./day) using a 100% (default) absorption rate and application rate should be converted to an equivalent oral dose (mg/kg/day).
>
> Then, the oral equivalent doses should be compared to the following NOAELs/LOAEL to calculate the MOEs.

1.3.7 Short-Term Inhalation (1 day to 30 days) Exposure

Study Selected: Chronic toxicity/carcinogenicity study- rats

MRID No.: 43303201

Executive Summary: See chronic RfD.

Dose and Endpoint for Risk Assessment: 9 mg/kg/day (LOAEL) based on increased methemoglobin. A NOAEL was not established.

Comments about Study/Endpoint: Methemoglobinemia is the principal toxicological effect of concern. This effect was seen at week 13 in rats and dogs (the first measurement period). There are no data for earlier time (i.e., days 1-89); therefore, this endpoint/dose was selected for this exposure scenario. An additional UF of 3 is applied to account for the lack of a NOAEL in this study.

1.3.8 Intermediate-Term Inhalation (1 month to 6 Months) Exposure

Study Selected: Chronic toxicity/carcinogenicity study- rats

MRID No.: 43303201

Executive Summary: See chronic RfD.

Dose/Endpoint for Risk Assessment: 9 mg/kg/day (LOAEL) based on increased methemoglobin. A NOAEL was not established.

Comments about Study/Endpoint: Methemoglobinemia is the principal toxicological effect of concern. This effect was seen at week 13 in rats and dogs (the first measurement period). This endpoint/dose observed at the 13 week observation period is appropriate for this exposure scenario. An additional UF of 3 is applied to account for the lack of a NOAEL in this study.

1.3.9 Long-Term Inhalation (Longer than 6 Months) Exposure

Study Selected: Chronic toxicity/carcinogenicity study- rats

MRID No.: 43303201

Executive Summary: See chronic RfD.

Dose and Endpoint for Risk Assessment: 9 mg/kg/day (LOAEL) based on increased methemoglobin and increased spleen weight in females, and observations of small seminal vesicles and prostates in males.

Comments about Study/Endpoint: The endpoint/dose is appropriate for this exposure scenario. An additional UF of 3 is applied to account for the lack of a NOAEL in this study.

1.3.10 Margins of Exposure for Occupational/Residential Risk Assessments

A MOE of 300 is required for short, intermediate, and long-term occupational dermal and inhalation exposure due to the use of a LOAEL. The acceptable MOEs for residential exposure will be determined by the FQPA SF committee.

1.3.11 Recommendation for Aggregate Exposure Risk Assessments

A toxicological endpoint was not identified for acute dietary risk assessment.

A common toxicological endpoint (methemoglobinemia) was selected for assessment of short and intermediate-term exposure by oral, dermal (oral equivalent), and inhalation (oral equivalent) routes. These routes can be aggregated for this scenario.

A common toxicological endpoint (increased methemoglobin and increased spleen weight in females, and observations of small seminal vesicles and prostates in males) was selected for long-term exposure by oral, dermal (oral equivalent), and inhalation (oral equivalent) routes. These routes can be aggregated for this scenario.

2 CLASSIFICATION OF CARCINOGENIC POTENTIAL

On May 9, 2001, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs met to evaluate the carcinogenic potential of propanil.

A summary of the CARC report (HED Doc. No. 014585) is as follows.

- **In Sprague-Dawley rats, there was a treatment-related increase in testicular adenomas** because: 1) There was a statistically significant positive trend and a statistically significant increase by pair-wise comparisons of the 600 and 1800 ppm dose groups with the controls for testicular interstitial cell adenomas in males (21% and 72%, respectively). 2) The incidences of these tumors in both dose groups were outside the range for the historical controls (0%-11%), and 3) These tumors were associated with an increased incidence of minimal interstitial cell hyperplasia. There was a difference of

opinion among the Committee members regarding whether the highest dose in male rats was adequate or excessive. Decreased body weight gains (30% decrease compared to controls at week 13) and a marked increase in methemoglobin level (MeHb; range: 84%-132% increase over the course of study) were considered by some Committee members to be indicative of excessive toxicity while the remaining members were of the opinion that despite these changes, there were no clinical signs of toxicity and survival of the animals was not affected by the treatment.

In females, there was a statistically significant positive trend and a statistically significant increase by pair-wise comparison of the 1800 ppm dose group with the controls for hepatocellular adenomas. The incidence of these tumors (13%) was outside the historical control range (0%-2%). The non-neoplastic changes in the liver were not severely adverse. **However the CARC determined that these tumors occurred at an excessively toxic dose based on decreased body weight gain (42% decrease compared to controls at week 13) and a marked increase in MeHb level (range: 106%-196% increase over the course of study).**

Although there was a borderline increasing trend, there was no significant increase by pairwise comparisons of the 600 and 1800 ppm dose groups with the controls for endometrial polyps. The increased trend was considered by the CARC to be skewed because not all animals in 200 and 600 ppm dose groups were microscopically examined. The changes in the uterine wall were not severely adverse. Moreover, the endometrial polyps are not tumors but are considered simply as a proliferative response of the endometrium to the damaging effects of steroid sex hormones.

The CARC concluded that the testicular tumors observed in male rats in this study were treatment-related. There was no treatment-related increase in tumors in female rats.

- **In Crl:CD-1 (ICR) BR mice, there was a treatment-related increase in commonly occurring malignant lymphomas in females** as evidenced by a statistically significant positive trend and a statistically significant increase by pair wise comparison of the 1000 ppm dose group with the controls for malignant lymphomas.. There was an increase in the incidence of malignant lymphomas from controls in the high dose group only. Usually the CARC prefers historical control data from the performing laboratory of same study duration and performed within two years of the study under review. In this case the historical control data from the performing laboratory was based on only <u>one</u> study of comparable duration of 24 months. The historical control data from 4 other studies from the same laboratory was for 18 months. Therefore, the CARC considered historical control data cited by the registrant, published between 1982-1995, from different laboratories which ranged from 0%-28%. The incidence of 17% at the high-dose was within this historical control range. Moreover, this tumor occurs spontaneously in this

sex and strain of mice. Therefore, the finding of malignant lymphomas at the high-dose was considered by the CARC to have a limited impact on the overall conclusion regarding the weight-of-the-evidence for the carcinogenic potential of propanil. No treatment-related tumors were observed in male mice.

The highest dose level tested was considered by the CARC to be adequate and not excessive because there were no treatment related adverse effects on the body weight gain, non-neoplastic histopathological findings or survival of the mice. However, there was an increase in MeHb levels, relative spleen weights and blue coloration of extremities in both sexes.

- A battery of pre-1991 acceptable Mutagenicity assays indicated that propanil was not genotoxic. No new studies were requested by the Committee.

- No mode of action studies related to the mechanism of tumor induction in rats or mice were available. Propanil causes anemia in laboratory animals. The mode of action for methemoglobinemia involves hydroxylation of propanil to form N-hydroxyaniline which oxidizes hemoglobin to form methemoglobin.

- **Classification of Carcinogenic Potential**

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified propanil into the category **"Suggestive evidence of carcinogenic potential by all routes of exposure, but not sufficient to assess human carcinogenic potential".** The decision was based on the following weight-of-the-evidence considerations: (1) Propanil induced testicular interstitial cell adenomas in male rats. The hepatocellular adenomas in female rats occurred only at an excessively toxic dose. The increase in commonly occurring malignant lymphomas in female mice added little to the overall weight of evidence for the carcinogenic potential of propanil. (2) Propanil was not mutagenic.

3. FQPA CONSIDERATIONS

3.1 Adequacy of the Data Base

The data base is adequate for FQPA assessment.

-- Acute delayed neurotoxicity study in hen: Not required.
-- Acute and subchronic neurotoxicity studies: Not available.
-- Developmental toxicity studies in Rats: Acceptable study available.
-- Developmental toxicity studies in Rabbits: Acceptable study available.

-- Two-Generation Reproduction Study in rats: Acceptable study available.
-- Developmental neurotoxicity study: Not available.

3.2 Neurotoxicity

No neurotoxicity studies have been submitted to the Agency. The HIARC considered the following findings are evidence of neurotoxicity:

In the chronic toxicity/oncogenicity study in rats (MRID 43303201), an increased incidence of axonal degeneration of the sciatic nerve (trace and minimal severity) was noted in female rats at the 104 week terminal sacrifice.

In the two-generation reproduction study in rats (MRID 44604301), significant delays in balanopreputial separation and in vaginal opening were noted in F1 male and female offspring; also, testicular sperm number and production were decreased in F1 males. These findings are highly suggestive of neuro-endocrine disruption. Caveats include that a lack of corollary body weight data from the day of endpoint achievement confounds the interpretation of these findings, and additionally, hormonal measurements in the two-generation reproduction study did not identify specific alterations in testosterone, luteinizing hormone (LH), or estrodiol levels in F0 males at study termination. Nevertheless, the delays in sexual development are supported by a number of other considerations, including: the presence of treatment-related testicular interstitial cell tumors in the rat chronic/oncogenicity study with propanil (often related to neuroendocrine disruptions), and the similarities between the reproductive toxicity profile of propanil to linuron and flutamide, two structurally-related chemicals with a demonstrated neuro-endocrine mode of action.

The following factors were also examined by the HIARC but, for the individual reasons given, were not considered to provide supportive evidence of neurotoxic potential.

In the prenatal developmental toxicity study in rats (MRID 00058588), an increased incidence of slightly dilated brain ventricles was observed in fetuses at the developmental LOAEL of 100 mg/kg/day. However, the HIARC stated that the dose-response characterization of this endpoint did not support an unequivocal relationship to treatment.

In the prenatal developmental toxicity study in rabbits (MRID 00058589), maternal clinical observations included loss of righting reflex and decreased motor activity. However, the HIARC noted that these findings were observed in only one doe that died prior to study completion and were interpreted as agonal, or at least related to severe systemic toxicity.

In the 24-month carcinogenicity study in mice (MRID 00155215) with 85.4% propanil,

bilateral retinal degeneration was observed in males and females at 180 ppm However, this finding was not confirmed in the 24-month carcinogenicity study in mice (MRID 43391701) with 97.1% propanil, and the HIARC believed that the retinal degeneration could have been related to impurities in the test substance instead of propanil *per se.*

3.3 Developmental Toxicity

Developmental toxicity study in rats

In an oral developmental toxicity study (MRID 00058588) propanil technical (85.4% a.i.) was administered to 25 presumed pregnant BLU:(SD)BR rats/dose group by gavage in corn oil vehicle (10 ml/kg body weight) at dose levels of 0, 0.8, 4.0, 20 or 100 mg/kg/day from presumed gestation days (GD) 6 through 15, inclusive.

The maternal toxicity LOAEL is 100 mg/kg/day, based on decreased body weight gain during treatment. The maternal toxicity NOAEL is 20 mg/kg/day. The developmental toxicity LOAEL is 100 mg/kg/day, based on decreased mean fetal weight and delayed ossification in the sternebrae and cervical vertebrae. The developmental toxicity NOAEL is 20 mg/kg/day.

This developmental toxicity study in the rat is classified acceptable/guideline and satisfied the guideline requirement for a rodent developmental toxicity study.

Developmental toxicity study in rabbits

In an oral developmental toxicity study (MRID 00058589), propanil technical (85.4% a.i.) was administered to 20 presumed pregnant (artificially inseminated) New Zealand white rabbits/dose group by gavage in corn oil vehicle (10 ml/kg body weight) at dose levels of 0, 4, 20 or 100 mg/kg/day from gestation days (GD) 6 through 18, inclusive.

The maternal toxicity LOAEL is 100 mg/kg/day, based on mortality, clinical signs of toxicity and weight loss during treatment. The maternal toxicity NOAEL is 20 mg/kg/day. The developmental toxicity LOAEL is 100 mg/kg/day, based on slightly decreased mean fetal weight. The developmental NOAEL is 20 mg/kg/day.

This developmental toxicity study in the rabbit is classified acceptable/guideline and satisfied the guideline requirement for a rabbit developmental toxicity study.

3.4 Reproductive Toxicity

Propanil (98.4% a.i) was administered to groups of 30 male and 30 female Crl:CD® (SD)BR rats in the diet at concentrations of 0, 60, 150, and 600 ppm for two generations

(MRID 44604301). Premating doses for the F0 males were estimated to be 0, 4, 11, and 43 mg/kg/day, respectively and for the F0 females were 0, 5, 13, and 51 mg/kg/day, respectively. Premating doses for the F1 males were estimated to be 0, 5, 13, and 53 mg/kg/day, respectively, and for the F1 females were 0, 6, 16, and 61 mg/kg/day, respectively. Animals were given test or control diet for at least 70 days then mated within the same dose group. All animals were exposed to test material in the diet and during lactation until sacrifice.

A tentative LOAEL for parental systemic toxicity is established at 60 ppm (LDT) (4 mg/kg/day for males and 5 mg/kg/day for females) based on increases in the severity of pigmented macrophages observed in spleens of F0 and F1 males and females in all dose groups. The tentative systemic parental toxicity NOAEL is <60 ppm (4 mg/kg/day for males and 5 mg/kg/day for females).

The LOAEL for reproductive toxicity is 600 ppm (43 mg/kg/day for males and 51 mg/kg/day for females), based on delayed vaginal perforation and balanopreputial separation in F1 adolescents, and on decreased mean testicular sperm count and production rate in F1 adult males. The reproductive toxicity NOAEL is 150 ppm (11 mg/kg/day for males and 13 mg/kg/day for females).

The LOAEL for offspring toxicity is 600 ppm (43 mg/kg/day for males and 51 mg/kg/day for females) based on reduced F1 and F2 pup weights, delayed sexual maturation in F1 males and females, and organ weight changes in F2 weanlings. The offspring NOAEL is 150 ppm (11 mg/kg/day for males and 13 mg/kg/day for females).

After reviewing the DER, the HIARC determined that the pigmented macrophages in the spleen observed at 60 and 150 ppm treatment groups are not considered adverse effects due to lack of dose-response relationship and the pronounced effects only were observed at 600 ppm group. Therefore, the HIARC determined the maternal NOAEL/LOAEL should be revised to 150/600 ppm, respectively, based on decreased body weight, body weight gain and food consumption. The HIARC also determined that this study should be classified as acceptable/guideline.

3.5 Additional Information from Literature Sources

Literature searches have been conducted and no additional neurotoxicity, developmental or reproductive toxicity was found.

3.6 Determination of Susceptibility

There was no evidence for quantitative susceptibility following *in vivo* exposures to rats and rabbits or following pre/post natal exposure to rats for two-generations. However,

there was evidence consistent with neuro-endocrine disruption (delayed vaginal opening and preputial separation) in the 2-generation reproduction study which indicated a qualitative susceptibility to the offspring.

3.7 Recommendation for a Developmental Neurotoxicity Study

There was evidence suggestive of neurotoxicity in the propanil data base. The findings included: Neuropathological lesions (sciatic nerve degeneration) in a rat chronic/carcinogenicity study. Evidence consistent with neuro-endocrine disruption (delayed vaginal opening and preputial separation) in the two-generation reproduction study in rats, and in the rat chronic/carcinogenicity study (increased incidence of testicular interstitial cell tumors); this evidence is supported by SAR considerations (the known neuro-endocrine mode of action of linuron, which is structurally related to propanil). A developmental neurotoxicity study in rats for propanil is required.

4. HAZARD CHARACTERIZATION

The toxicological database for propanil is considered minimally adequate for hazard characterization. Propanil has low acute toxicity, with toxicity categories of III (oral) and IV (dermal, inhalation, and primary skin irritation); no dermal sensitization was observed, however, primary eye irritation was observed in rabbits (toxicity category II). For non-acute exposures, administration of propanil to different species for varying lengths of time leads characteristically to the development of methemoglobinemia. The resulting methemoglobinemia results in the development of hemolytic anemia, which is associated by decreases in some or all of the following parameters: hemoglobin, RBC count and packed cell volume. Hematological and histopathological evaluations also revealed Heinz bodies in RBCs and hemosiderin deposits in the spleen and kidneys.

The hematological effects of propanil were most evident in the chronic toxicity studies in the rat and dog, although there is evidence that the mouse can also be affected. In a 2-year dietary study in the rat, a dietary level of about 80 mg/kg/day caused a decrease in overall growth and a relative increase in the weight of the spleen and liver in female rats and of the testes in males. Feed consumption, growth, and hemoglobin levels in rats were reduced at daily doses of about 180 mg/kg/day over 3 weeks.

The chronic toxicity of propanil has been evaluated in 2-year feeding studies in both the rat and dog. Body weights were measured weekly during the entire study. In the rat study, after one week of treatment, a significant decrease in body weight gain was observed at 600 ppm in males (59% of control) and females (53% of control). These decreases persisted throughout the entire study, with females showing a consistently lower body weight gains (68 to 76%) than males (82 to 93%). The decreases in body weight gains correlated to some degree with decreased food consumption.

In the dog study, a dose-dependent increase in methemoglobin formation was observed, and graded as moderate to severe at the two highest dose levels (1600 and 3200 ppm). Hematological evaluation also revealed decreases in RBC count, hemoglobin, packed cell volume and mean cell hemoglobin concentration; reticulocyte smears showed increased incidences of Heinz body formation. Brown pigmentation (hemosiderin) was found in the bone marrow, kidney and liver of both sexes; the grade and incidence of hemosiderin deposits were dose-related.

Propanil was not mutagenic in bacteria or in cultured mammalian cells. There was also no indication of a clastogenic effect up to toxic doses *in vivo*. Propanil, however, did cause DNA damage in a DNA repair-deficient strain of *B. subtilis*. However, the relevance of this positive result in bacteria is unclear since DNA damage was not manifested as point mutations in microbial systems or mammalian cells, mitotic recombinations in yeast, DNA damage in mammalian cells, or chromosomal aberrations in whole animals. In oncogenicity studies in the rat and mouse, tumors were observed, however, sex and species differences were noted. In the mouse oncogenicity study, a significant increase in malignant lymphomas were observed in the spleen of females. In the combined chronic toxicity/oncogenicity study in rats, testicular adenomas were observed in 16% and 58% of males at 600 and 1800 ppm, respectively, compared to 6% in both control and low-dose groups. In female rats, the incidence of endometrial polyps was observed at 600 (15%) and 1800 (13%) ppm, compared to 4% for control and 8% at 200 ppm. The HED CARC has classified propanil into the category "suggestive evidence of carcinogenic potential by all routes of exposure, but not sufficient to assess human carcinogenic potential".

Although no mechanistic studies have been submitted to the agency, open literature publications addressed the mode of action of propanil. The ability of propanil and its metabolites to induce methemoglobinemia was evaluated in both *in vitro* and *in vivo* experiments. Using a rat microsome preparation, propanil was metabolized to 3,4-dichloroaniline (DCA) via acrylamidase-catalyzed hydrolysis; 2'-hydroxy-3,4-dichloroaniline (N-OH-DCA)and 6-hydroxy-3,4-dichloroaniline (6-OH-DCA) were also identified. Of these three metabolites, N-OH DCA and 6-OH DCA produced methemoglobin in a dose-dependent manner in rat RBC suspensions. Of the two methemoglobin-inducing metabolites, N-OH-DCA was at least 10 times greater than 6-OH-DCA. Following propanil administration (1.0 mmole/kg (218 mg/kg)) to rats showed that both dichloroanaline and N-hydroxy-3,4-dichloroanaline were found in the blood.

Reproductive and developmental toxicity - Other than slightly decreased fetal body weights (with or without accompanying delays in skeletal ossification) there was no apparent effect of *in utero* propanil exposure on the morphological development of the fetuses in the prenatal developmental toxicity studies in rats and rabbits. In the two-generation reproduction study in rats, delayed vaginal perforation and balanopreputial separation was observed in F1 adolescents, and decreased mean testicular sperm count

and production rate was noted in F1 adult males. These findings are highly suggestive of neuroendocrine disruption, although hormonal measurements in the two-generation reproduction study did not identify specific alterations in testosterone, luteinizing hormone (LH), or estrodiol levels in F0 males at study termination. Nevertheless, the delays in sexual development are supported by a number of other considerations, including: the presence of treatment-related testicular interstitial cell tumors in the rat chronic/oncogenicity study with propanil (often related to neuroendocrine disruptions), and the similarities between the reproductive toxicity profiles of propanil to linuron and flutamide, two structurally-related chemicals with a demonstrated neuro-endocrine mode of action affecting the hypothalamic-pituitary-testicular (HPT) axis. (In rat studies, linuron has been shown to 1) delay sexual maturation, 2) cause abnormalities in male reproductive organs and result in alterations to spermatogenesis, and 3) result in significant incidences of Leydig cell tumors after prolonged exposure.)

5. DATA GAPS

The HIARC determined that a 28-day inhalation study is required to address the concern for inhalation exposure potential based on the use pattern. The Registrant can follow the 90-day inhalation study protocol but cease exposure at 28 days. The HIARC also determined that a developmental neurotoxicity study is required. In addition, the HIARC recommended a 30-day oral study in rats with methemoglobin measurements at days 1, 5, 7, 14, 21, and 30 for potential hazard identification. There is evidence in the published literature suggesting that propanil is a potential immunotoxic compound. Therefore, the HIARC recommended the Registrant to conduct a guideline immunotoxicity study (OPPTS 870.7800) or submit a literature search to better characterize its immunotoxic potential.

6. ACUTE TOXICITY

Acute Toxicity of Propanil

Guideline No.	Study Type	MRIDs #	Results	Toxicity Category
81-1	Acute Oral	41360801 008722	LD_{50} = 1080 mg/kg (M&F)	III
81-2	Acute Dermal	41360901 008722	LD_{50} >2000 mg/kg	III
81-3	Acute Inhalation	41415501 008423	LC_{50} >6.1 mg/L	IV

81-4	Primary Eye Irritation	41360501 008430	Iritis, conjunctivitis present in all rabbits, cleared by day 14; corneal opacity cleared by 4 days	II
81-5	Primary Skin Irritation	41360601 008430	Slightly irritating P.I. = 0.2/4.0	IV
81-6	Dermal Sensitization	41360401 008430	Nonsensitizer	N/A
81-8	Acute Neurotoxicity	N/A	Not available	N/A

7. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	There is no appropriate endpoint attributed to a single dose was identified. Therefore, an acute RfD was not established.		
Chronic Dietary	LOAEL = 9 UF = 300	Increased methemoglobin and increased spleen weight in females, and small seminal vesicles and prostates in males.	Chronic toxicity/ carcinogenicity study in rats
		Chronic RfD = 0.03 mg/kg/day	
Cancer	Suggestive evidence of carcinogenic potential by all routes of exposure, but not sufficient to assess human carcinogenic potential.	(1) Propanil induced testicular interstitial cell adenomas in male rats. The hepatocellular adenomas in female rats occurred only at an excessively toxic dose. The increase in commonly occurring malignant lymphomas in female mice added little to the overall weight of evidence for the carcinogenic potential of propanil. (2) Propanil was not mutagenic.	Carcinogenicity study in rats and mice
Incidental Oral; short- and Intermediate-Term	LOAEL= 9	Increased methemoglobin.	Chronic toxicity/ carcinogenicity study in rats
Dermal; Short-Intermediate-Term[a]	LOAEL= 9	Increased methemoglobin.	Chronic toxicity/ carcinogenicity study in rats
Dermal; Long-Term[a]	LOAEL= 9	Increased methemoglobin and increased spleen weight in females, and small seminal vesicles and prostates in males.	Chronic toxicity/ carcinogenicity study in rats
Inhalation; Short-, Intermediate-Term[b]	LOAEL= 9	Increased methemoglobin.	Chronic toxicity/ carcinogenicity study in rats
Inhalation; Long-Term[b]	LOAEL = 9	Increased methemoglobin and increased spleen weight in females, and small seminal vesicles and prostates in males.	Chronic toxicity/ carcinogenicity study in rats

[a] An oral endpoint was used for dermal exposure: dermal absorption factor of 20% of oral exposure shall be used.
[b] An oral endpoint was used for inhalation exposure: inhalation exposure assumed equivalent to oral exposure.